ATTORNEY DOCKET NO. T1530-00094
(formerly 100337.54260US)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent Application No. 10/628,464

Filed: July 29, 2003

Art Unit: 1646 Confirmation No. 4793

Title: IDENTIFICATION OF A NOVEL BITTER TASTE RECEPTOR, T2R76

AFFIDAVIT BY MARK J. ZOLLER , Ph. D.

I, Mark Zoller, declare and state as follows:

(1) That I am an inventor of the above-identified application..

(2) That I am currently employed. as the Chief Scientific Officer and Senior Vice President at Senomyx Inc., the Assignee of this patent application..

(3) That I reviewed the Office Action dated March 30, 2005 in the above-identified patent application relating to the hT2R76 a novel member of the T2R taste receptor family.

(4) That based thereon I understand that the Examiner has initially concluded that the as-filed specification contains insufficient evidence to reasonably that hT2R76 is a bitter taste receptor. I respectfully disagree.

(5) That experiments have been conducted at Senomyx Inc. under my supervision which have confirmed that hT2R76 encodes a bitter taste receptor that specifically binds to bitter ligands including PROP a bitter ligand specifically identified in our as-filed patent application

(6) (See example 6 of our patent application which lists 6 bitter ligands as being putative bitter ligands for hT2R76 including PROP.)

(7) That more specifically experiments were conducted using cell-based assays that detect changes in intracellular calcium concentration. In brief, using essentially the same fluorescent detection methods described in our patent application, human embryonic cells (HEK-293 cells) are initially seeded into 48-cell culture plates. 24 hours late, these cells are transiently transfected with a plasmid containing the hT2R76 nucleic acid sequence disclosed in our patent application along with a plasmid encoding a G protein (G16gust44). Another 24 hours later these cells are incubated with a fluorescent dye specific for calcium (Fluo-4; Molecular Probes) that provides a fast, simple and reliable fluorescent-based method for detecting changes in calcium concentration within the cell. [If a ligand specifically activates hT2R76, this elicits a signaling cascade, which leads to the activation of PLC and a subsequent increase in intracellular concentration. This increase in intracellular calcium concentration affects the fluorescent properties of the calcium specific dye within the cells. These changes may be monitored using the fluorescent microscopy imaging methods disclosed in our patent application using specifically designed software. (Imaging Workbench, Axon).]

(8) That using these methods we screened HEK-293 cells expressing the above-identified hT2R76 sequence with a number of bitter ligands (including PROP, Brucine (a bitter alkaloid found in Strychnos seeds), L-tryptophan, salicin, and N-phenylthiourea.)

(9) That using these methods we observed that the PROP and Brucine bitter ligands both specifically activated hT2R76 expressed in HEK-293 cells, resulting in detectable changes in intracellular calcium levels (increase in fluorescence). By contrast, the other (control) bitter

ligands, including L-tryptophan, salicin, and phenylthiourea had no effect on intracellular calcium levels as evidenced by no detectable changes in fluorescence. (The results of these calcium imaging experiments are contained in Figure 2 attached to this affidavit.)

(10) That in my opinion the results of these experiments confirm what we reasonably anticipated on filing this patent application, i.e., that hT2R76 (SEQ ID NO:1) encodes a human bitter taste receptor which when screened against bitter ligands using the methods described herein and in our patent application would be shown to specifically respond to known and available bitter ligands such as PROP and Brucine. [Brucine is a well known bitter toxic alkaloid expressed in Strychnos seeds with a bitter taste threshold of 0.01 mM. Similarly, PROP is a well known bitter compound that elicits a bitter taste threshold of 0.01mM for PROP tasters].

(11) All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like may jeopardize the validity of the application or any patent issuing thereon.

9/28/05
Date

Mark J. Zoller

ATTORNEY DOCKET NO. T1530-00094
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In re U.S. Patent Application No. 10/628,464

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Art Unit: 1646 Confirmation No. 4793

Title: IDENTIFICATION OF A NOVEL BITTER TASTE RECEPTOR, T2R76

AFFIDAVIT BY ROBIN L. TESKIN

I, Robin L. Teskin, declare and state as follows:

(1) That I am a registered patent attorney and outside patent counsel for Senomyx Inc. and in that capacity drafted the above-identified application..

(2) That in order to prepare this draft patent application I was provided an invention disclosure reviewed and signed by the inventors of the above-identified patent application. This invention disclosure is attached as an Exhibit to this Affidavit..

(3) That I reviewed the Office Action dated March 30, 2005 in the above-identified patent application relating to the hT2R76 a novel member of the T2R taste receptor family.

(4) That based thereon I understand that the Examiner has initially concluded that the claims are anticipated or rendered obvious by a PCT application WO/057309, entitled "Novel G protein-Coupled Receptor Protein and DNA Thereof". This PCT patent application has a publication date of July 25, 2002 which predates the earliest priority date of the instant patent

application which claims priority to and incorporates by reference in its entirety provisional patent application Serial No. 60/398,727 filed on July 29, 2002.

(5) That this reference is not prior art to the subject invention as evidenced by the invention disclosure executed by all of the inventors of this patent application prior to July 25, 2002 which I moreover received prior to July 25, 2002 and which document establishes unequivocally that the present inventors were in possession of the claimed invention prior to the publication date of WO/057309. Particularly this invention disclosure contains the hT2R76 nucleic acid sequence and corresponding polypeptide sequence as well as disclosing its identity as a bitter taste receptor and its use in assays for identifying bitter taste modulators as claimed in the patent application at issue herein. [The undersigned notes that this invention disclosure has been redacted in several places to protect some confidential information of the present Assignee as permitted but that the execution dates of the signatures contained thereon clearly establish that Senomyx's discovery of a new bitter receptor hT2R76 as claimed herein predates the publication date of the cited PCT reference.]

(6) That based thereon, the undersigned respectfully submit that the rejection based on PCT WO 02/057309 should be withdrawn as this document is not available as prior art against the claimed invention.

(7) All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like may jeopardize the validity of the application or any patent issuing thereon.

September 30, 2005
Date

Robin L.. Teskin

Senomyx, Inc.

Senomyx File No.: 2002-045

Date Received: 7/2/02

Initials: SCD

RECORD OF INVENTION

This document will constitute a complete disclosure of this invention. In order to expedite processing, all sections must be completed or noted as N/A (not applicable).

Submit this original executed by all inventors and an electronic version (email or disk) to the Legal Department.

1. TITLE OF INVENTION: **Identification of a novel putative human bitter taste receptor, hT2R76.**

2. INVENTOR(S): (Attach copies of current CVs if possible)

(a) Name: **Jon Elliot Adler**
Title _____ Telephone _____
Residence Address _____
Country of Citizenship _____

Signature_____ Date _____

(b) Name: **Huixian Tang**
Title _____ Telephone _____
Residence Address _____
Country of Citizenship _____

Signature_____ Date _____

(c) Name: **Alexey Pronin**
Title _____ Telephone _____
Residence Address _____
Country of Citizenship _____

Signature_____ Date _____

For additional information, please contact: _____

Please identify any inventor(s) named above who are not employed by Senomyx, Inc. and indicate whether they are students, employees of other companies or institutions, etc.: _____

3. DESCRIPTION OF INVENTION. Describe the invention fully and completely following the outline given below. Use additional sheets as necessary and include descriptive materials such as sketches, drawings and/or photographs that may promote a better understanding of the invention. (Rough artwork, flow charts, Polaroid photographs and penciled graphs and photocopies of laboratory notebook pages are satisfactory.)

(a) Classify the invention as one or more of the following: a new process, composition of matter, a device, one or more products, a new use or an improvement to an existing product or process.

Composition of matter (gene and protein sequences).

(b) Give a detailed description of the invention, including materials, mechanics, synthesis steps, characteristics, etc.

Field of the invention.

The present invention relates to and has among its objects the reduction of bitterness due to bitter materials present in food, beverages and pharmaceutical preparations.

Summary of the invention.

We have identified a novel gene for a human bitter taste receptor in the human genome sequence database. Novel hT2R member, hT2R76 was initially identified by reiterated sequence search of DNA sequence databases with previously described hT2R sequences. Full-length open reading frame encoding hT2R76 was then isolated by PCR amplification of genomic DNA, and its structure was confirmed by nucleotide sequencing. The amino acid sequences of receptor encoded by the gene was derived by conceptual translation of the corresponding open reading frame. Comparison of this protein sequences to all known proteins in the public sequence databases using BLASTP algorithm reveals its strong homology to the members of the mammalian bitter receptor family. The hT2R76 gene is located on human chromosome 7, intronless and encodes putative receptor protein 318 amino acid residues in length.

A bridge overlap PCR extension technique was used to generate rhod-hT2R76 chimeras, which contain the first 38 amino acids of bovine rhodopsin

in frame with human T2R76 coding sequences as described (Chandrashekar et al., 2000). The chimeric rhod-hT2R76 gene was then cloned into pFastBac-1 vector and baculoviruses containing rhodopsin-tagged hT2R76 was produced using Bac-to-Bac system (Invitrogene). Expression of hT2R76 was confirmed by immunoblotting using anti-rhodopsin tag antibodies (B6-30). Sf9 cells infected with hT2R76-encoding baculovirus produced a protein of the expected molecular weight (~35 kDa).

Example 1.

hT2R76 gene sequence

ATGAATGGAGACCACATGGTTCTAGGATCTTCGGTGACTGACAAGAAGGCCATCATCTTGGTTACCATTTTACTCCT
TTTACGCCTGGTAGCAATAGCAGGCAATGGCTTCATCACTGCTGCTCTGGGCGTGGAGTGGGTGCTACGGAGAATGT
TGTTGCCTTGTGATAAGTTATTGGTTAGCCTAGGGGCCTCTCGCTTCTGTCTGCAGTCAGTGGTAATGGGTAAGACC
ATTTATGTTTTCTTGCATCCGATGGCCTTCCCATACAACCCTGTACTGCAGTTTCTAGCTTTCCAGTGGGACTTCCT
GAATGCTGCCACCTTATGGTCCTCTACCTGGCTCAGTGTCTTCTATTGTGTGAAAATTGCTACCTTCACCCACCCTG
TCTTCTTCTGGCTAAAGCACAAGTTGTCTGGGTGGCTACCATGGATGCTCTTCAGCTCTGTAGGGCTCTCCAGCTTC
ACCACCATTCTATTTTTCATAGGCAACCACAGAATGTATCAGAACTATTTAAGGAACCATCTACAACCTTGGAATGT
CACTGGCGATAGCATACGGAGCTACTGTGAGAAATTCTATCTCTTCCCTCTAAAAATGATTACTTGGACAATGCCCA
CTGCTGTCTTTTTCATTTGCATGATTTTGCTCATCACATCTCTGGGAAGACACAGGAAGAAGGCTCTCCTTACAACC
TCAGGATTCCGAGAGCCCAGTGTGCAGGCACACATAAAGGCTCTGCTGGCTCTCCTCTCTTTTGCCATGCTCTTCAT
CTCATATTTCCTGTCACTGGTGTTCAGTGCTGCAGGTATTTTTCCACCTCTGGACTTTAAATTCTGGGTGTGGGAGT
CAGTGATTTATCTGTGTGCAGCAGTTCACCCCATCATTCTGCTCTTCAGCAACTGCAGGCTGAGAGCTGTGCTGAAG
AGTCGCCGTTCCTCAAGGTGTGGGACACCTTGA

hT2R76 protein sequence

MNGDHMVLGSSVTDKKAIILVTILLLLRLVAIAGNGFITAALGVEWVLRRMLLPCDKLLVSLGASRFCLQSVVMGKT
IYVFLHPMAFPYNPVLQFLAFQWDFLNAATLWSSTWLSVFYCVKIATFTHPVFFWLKHKLSGWLPWMLFSSVGLSSF
TTILFFIGNHRMYQNYLRNHLQPWNVTGDSIRSYCEKFYLFPLKMITWTMPTAVFFICMILLITSLGRHRKKALLTT
SGFREPSVQAHIKALLALLSFAMLFISYFLSLVFSAAGIFPPLDFKFWVWESVIYLCAAVHPIILLFSNCRLRAVLK
SRRSSRCGTP

(c) Discuss the existing technology with emphasis on limitations and disadvantages.

Description of the Prior Art.

One of the basic taste modalities that humans can recognize is bitter. The physiology of bitter taste is very poorly understood. However, recent studies started to shed light on the biology of taste (Lindemann, 2001). It is believed that many bitter compounds produce bitter taste by interacting with cell surface receptors. These receptors belong to the family of seven transmembrane domains receptors that interact with intracellular G proteins (G protein-coupled receptors, or GPCRs). A novel family of GPCRs, termed T2Rs, was recently identified in humans and rodents (Adler et al., 2000; Chandrashekar et al., 2000; Matsunami, 2000). Several lines of evidence

suggested that T2Rs mediate response to bitter compounds. First, T2R genes are specifically expressed in subset of taste receptor cells of the tongue and palate epithelia. Second, the gene for one of the human T2Rs (hT2R1) is located in a chromosomal locus that is linked to sensitivity to bitter compound 6-n-propyl-2-thiouracil in humans (Adler et al., 2000). Third, one of the mouse T2Rs (mT2R5) is located in a chromosomal locus that is linked to sensitivity to bitter compound cycloheximide in mice. It was also shown that mT2R5 can activate gustducin, G protein specifically expressed in taste cells and linked to bitter stimuli transduction (Wong et al., 1996). Gustducin activation by mT2R5 occurs only in response to cycloheximide (Chandrashekar et al., 2000). Thus, it has been proposed that mT2R family mediates bitter taste response in mice, whereas hT2R family mediates bitter taste response in humans. Only one human T2R was suggested as having identified bitter ligand - hT2R04 was shown as being activated by denatonium (Chandrashekar et al., 2000). Total of 24 human genes were previously identified. Sequences of hT2Rs are described in scientific publications (Adler et al., 2000; Matsunami, 2000) and Patent Applications (Zuker 2001; Adler 2001).

(d) From the description in 4(b), expand on the novel and unusual features that distinguish this invention from the existing technology. Discuss the problems the invention solves and the advantages the invention has over existing technology.

(e) Comment on possible applications/uses of the invention (immediate and/or future).

Possible applications/uses of the invention.

In addition to other taste and flavor components, some foods and food ingredients have a bitter taste. Some pharmaceutical compounds also have bitter taste if taken orally. In many cases bitter taste is undesirable and may prevent consumers from utilizing this kind of ingredients. Therefore, it might be desirable to reduce a bitter taste of food and pharmaceutical compounds. Currently there is no proven way to efficiently block a bitter taste. In most cases a bitter taste is masked by the addition of sweet compounds, such as sugar. However, the addition of a sweetener may undesirably alter a food flavor and increase calorie intake. Artificial super sweeteners provide a low calorie alternative to sugar. However, some sweeteners, such as saccharin, have an unpleasant bitter taste to many people.

Identification of a putative family of human bitter receptors may allow developing of compounds, which specifically block a bitter taste of known bitter substances. The first step in this process is identification of specific hT2Rs that are activated by a particular bitter molecule. Next, the identified

bitter compound-hT2R pair can be used to discover molecules that block activation of the receptor (antagonists). Because receptor antagonist works by competing with the agonist for the same binding site, the same antagonist can block receptor activation by multiple agonists. Thus, a compound that reduces a bitter taste of one bitter ingredient may also reduce a bitter taste of other bitter substances, which work through the same hT2R.

Here we described identification of a novel human bitter receptor gene that has amino acid sequence distinctly different from all previously known bitter receptors, which suggests its different specificity in tastant recognition. Therefore, this novel receptor and its gene can be used in developing detection systems and assays for chemically distinct types of tastants not recognized by the known receptors, as well as for diagnostic and research purposes. The novel receptor can be used to identify molecules that specifically block activity of hT2R76 and thus are able to reduce a bitter taste of a specific subset of bitter substances found in food, beverages and pharmaceutical preparations. Addition of such compounds to products may improve their taste and make them more desirable to consumers.

(f) Describe any disadvantages or limitations of the invention. Can they be overcome? How?

(g) If this is a joint invention, indicate what contribution each inventor made.

4. SENOMYX PATENT FAMILY GROUP:

☐ T1R Receptor	☐ ENAC (salt channel)	☐ VNO
☒ T2R Receptor	☐ Umami	☐ Other: _____
☐ Sour	☐ Olfactory Receptor	

5. DATES AND PLACES OF INVENTION:

(a) Earliest date and place invention was conceived.

(b) Date and present location of first written description, sketch, drawing or photo.

(c) Date and place of completion of first reduction to practice of the invention. (Completion of model or full size device.)

(d) Date and place of first test or operation of invention.

(e) What is the present state of development?

6. PUBLICATION/PUBLIC DISCLOSURE. Under 35 USC §102(b), a valid patent cannot be obtained if "the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States." In addition, because most foreign countries require absolute novelty as a condition of patentability, the right to obtain patent protection in countries such as West Germany and Japan may be lost upon publication.

(a) List all publications (theses, reports, preprints, reprints, abstracts, news releases, etc.) pertaining to the invention. Please attach copies and note publication dates.

(b) Indicate the date, place and circumstances of first public disclosure.

(c) Describe in detail any plans for disclosure of this invention in the near future.

(1) Manuscripts

(a) List all manuscripts in preparation for publication with proposed submission dates.

(b) List submitted manuscripts. Include date of submission, journal or publication title and approximate date of publication.

(2) Conferences/Seminars

(3) Other

(d) List any related patents or publications which you believe to be pertinent to this invention. Please attach a copy of each reference.

7. SPONSORSHIP (*other than Senomyx, Inc.*)

(a) Was this invention developed in the course of duties at any company or university and/or with the use of a university facility?

☐ YES ☒ NO

(b) List the source(s) of funding under which this invention evolved (i.e.,

Governmental agencies, industrial sponsors, university sponsors, foundations, etc.)

Sponsor	Award No.	Funding Period	P.I.

CLAIMS:

Having described our invention, we claim:

1. It is an object of the invention to provide a new human gene, hT2R76, active in bitter taste perception.

2. It is yet another object of the invention to provide nucleic acid sequences or molecules that encode hT2R76, fragments or variants thereof.

3. A process of using hT2R76 to identify molecules that reduce bitter taste of bitter substances employing cell-based, GTPγS binding or any other assay.

Cited literature.

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJ, Zuker CS. A novel family of mammalian taste receptors. *Cell* 2000, 100(6): 693-702

Adler E. T2R taste receptors and genes encoding same. 2001, Patent application WO 01/77676 A1

Chandrashekar J, Mueller KL, Hoon MA, Adler E, Feng L, Guo W, Zuker CS, Ryba NJ. T2Rs function as bitter taste receptors. *Cell* 2000, 100(6): 703-11

Lindemann B. Receptors and transduction in taste. *Nature* 2001, 413(6852): 219-25

Matsunami H, Montmayeur JP, Buck LB. A family of candidate taste receptors in human and mouse. *Nature* 2000, 404(6778): 601-4

Wong GT, Gannon KS, Margolskee RF. Transduction of bitter and sweet taste by gustducin. *Nature* 1996, 381(6585): 796-800

Zuker C, Adler E, Ryba N, Mueller K. T2R, a novel family of taste receptors. 2001, Patent application WO 01/18050 A2